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Analysis of Relationship between Tumor Necrosis Factor Alpha Gene (G308A Polymorphism) with Preterm Labor

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Original Article

ABSTRACT

Background: Increased concentrations of tumor necrosis factor alpha (TNF-α) in blood and amniotic fluid are observed in women with preterm delivery (PTD) and TNF-α mutations at −308 position are associated with higher expression of this gene. Therefore, we compared the frequency of G308A transition in the promoter region of TNF-α gene of women and neonates delivered preterm with the normal subjects.

Methods: This cross-sectional study was performed on 135 mothers who were referred for delivery. According to the gestational age, mothers and their neonates were allocated to the case (preterm, 64 subjects) and control (term, 71 subjects) groups. Using the polymerase chain reaction, restrictive fragment length polymorphism (RFLP), genotyping was performed on both maternal peripheral blood and cord blood samples to determine single nucleotide polymorphism in the promoter region of TNF-α gene at –308.

Results: Two mothers in the case group, one mother in the control group and one neonate in the case group had genotyping assays (GA) mutation. All other subjects had normal GG genotype. Frequency of GA mutation was not significantly different between two groups ($P = 0.47$).

Conclusions: There is no significant association between PTD and either maternal or fetal TNF-α −308 polymorphism and frequency ofGAmutation is not significantly increased in mothers and neonates delivered preterm. It means that the presence of this mutation by itself does not modify the overall risk of PTD. Investigations on the combination of various polymorphisms indifferent genes are recommended to achieve more accurate results.

Keywords: Genetics, mutation, polymorphism, preterm birth, tumor necrosis factor alpha

INTRODUCTION

Preterm delivery (PTD) is a major public health problem world-wide, which may lead to severe complications such

as cerebral palsy, chronic respiratory disease, blindness and neuro-developmental delay. It is also one of the main causes of perinatal and neonatal death[1-3] and imposes a large economic burden on the health-care system.[4,5]

Increased rate of PTD in women who had PTD, high probability of PTD in women with a previous history of PTD and studies of pregnancy outcomes in twins suggest that genetic factors may be important determinants of PTD.[2,6]

In addition, previous studies suggest that the inflammation caused by subclinical genital tract infection may be associated with PTD.[7,8]

Increased concentration of proinflammatory cytokines has been found in amniotic fluid, maternal and fetal blood of a significant number of women with PTD.[9-15] Tumor necrosis factor-α (TNF-α) is one of these a proinflammatory cytokine that can trigger a cascade of events and lead to PTD.[2,16] Expression of cytokines such as TNF-α is also under genetic control.[17]

It has been reported that a genetic variant single nucleotide polymorphism (SNP) in the promoter region of TNF-α gene at −308 (a G/A transition resulting in TNF-αamino acid (AA) or genotyping assays (GA) genotypes) may be associated with increased activation of the TNF-α gene compared with the GG genotype.[18-20]

Given the common genetic background of PTD and TNF-α expression, a number of studies have been performed to investigate the role of genetic variation at the level of SNPs within genes that regulate the inflammatory response, to determine whether there is an association between this genetic variations and predisposition to PTD in women or not. Although large-scale studies have been conducted to assess the association of the G308A polymorphism of TNF-α with PTD, no strong convincing evidence of association has been found and this hypothesis remains controversial.[2]

This study was designed to search for an association between PTD and maternal or fetal SNP in TNF-α −308 gene.

METHODS

Study population and design

After approval of the study by the ethic committee of Shahrekord University of Medical Sciences and obtaining informed consent, this cross-sectional study was performed on mothers who were referred to the Hajar hospital for delivery and their infants. This investigation was performed in Shahrekord, Iran, between March 2010 and April 2012.

Using convenience sampling method, pregnant women aged between 18 and 35 years with a gestational age (GA) between 28 and 42 weeks were included in this study.

Exclusion criteria were any history of cervical incompetency, polyhydramnios, preeclampsia, vaginal bleeding during pregnancy, fetal abnormality, cord or placental abnormalities, psychological problems, diabetes or other chronic cardiac, kidney, pulmonary or collagen vascular diseases.

According to the gestational age at the time of delivery, women who met the study criteria were allocated to term (control) and preterm (case) groups.

Last menstrual period date confirmed by ultrasound dating was used to determine GA genotype. Mothers with preterm contractions (presence of at least 2 regular uterine contractions per 10 min combined with documented cervical changes at GA of $< 36\frac{0}{7}$ weeks) at the time of hospital admission for delivery were assigned to the case group and mothers with the normal labor and delivery (GA $> 37\frac{0}{7}$ weeks) were assigned to the control group.

Subjects with GA between $36\frac{1}{7}$ and $36\frac{6}{7}$ weeks were excluded to avoid the potential ambiguity of GA in this range.

Finally, a total of 135 women and their infants were recruited (71 in the term group and 64 in the preterm group).

Blood sampling and laboratory assays

For genotyping, a maternal peripheral blood sample and a fetal cord blood sample were taken. Blood samples of 5 ml were collected in 15 ml falcon tubes already containing 60 μl ethylene diamine tetra acetic acid (0.5 molar). All blood samples were frozen at −20°C for 24 h before polymerase chain reaction (PCR) and genotyping.

The sequence of the primer was forward (5'-TCCAGACTTTGAGACACG-3') and reverse (5'-GGGCGATTACAGACAACT-3').

According to the standard protocols, deoxyribonucleic acid (DNA) was extracted from blood samples by the phenol-chloroform method using lysis buffer (Tris-HCl10 ml, sucrose, $MgCl_2$, 5ml, tryptone X10, 10 ml.

The PCR was carried out in 0.25 ml tubes containing 100 ng sample of DNA, 4 mmol 1x-PCR buffer (500 mmolKCl, Tris-HCl with PH: 8.4 and 200 mmolAmS), $MgCl_2$, 3 mmol, dNTPS: Deoxy ribo Nucleotide Triphosphates, (dTTP = Deoxy Thymidine Triphosphate, dATP Deoxy Adenosine Triphosphate, dGTP Deoxy Guanosine Triphosphate, dCTP Deoxy Cytidine Triphosphate) 0.2 mmol/l, DNA Taq polymerase 0.5 mL and primers Cycling was performed at 95°C for 5 min; then for 34 cycles of 94°C, for 50 s (denaturation), 60°C for 50 s (annealing) and 72°C for 30 s (extension); and finally for 5 min at 72°C (final extension).

Then, 2.5 µl of PCR amplification products was mixed with 0.5 µl of loading dye and was electrophoresed on 5% polyacrylamide gels. Silver staining was used to detect proteins. In order to digestion of PCR products, they were mixed with 1 µl of Tango buffer and 0.5 µl of NCo1 enzyme for 3 h at 37°.

At this stage, 5 µl of digested products was mixed with 4 µl of loading dye and the sample was assessed using the polyacrylamide gel electrophoresis and silver staining [Figure 1]. Finally, TNF-α −308 genotype and alleles were determined.

Statistical analysis

Data was analyzed by the Statistical Package for the Social Sciences (SPSS) 20.0 (SPSS Inc., Chicago, IL, USA) using independent *t*-test and Chi-square. $P < 0.05$ were considered statistically significant.

RESULTS

Baseline data

Mean of GA and neonatal birth weight was significantly lower in the case group. However, there was no significant difference between 2 groups regarding other baseline characteristics [Table 1].

TNF-α–308 polymorphism

Only 1 (1%) mother in the control group and 2 (3%) mothers in the case group had GA genotype, which was not significantly different ($P = 0.46$).

All cord samples in both groups had GG genotype except 1 (1.5%) sample in the case group,

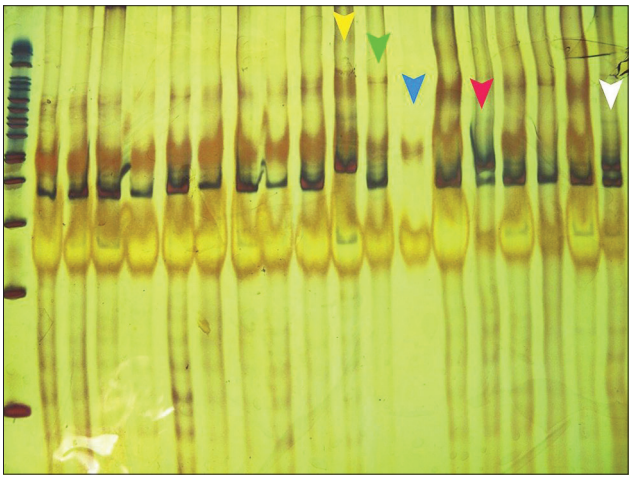

Figure 1: Polyacrylamide gel electrophoresis demonstrating allelic variants of the tumor necrosis factor alpha −308 polymorphism. White arrow: Mutation, red arrow: Mutation, blue arrow: Negative control, green arrow: Digested, yellow arrow: Positive control (uncut)

Table 1: Comparison of baseline characteristics between 2 groups

Baseline characteristics	Case group (*n*=64)	Control group (*n*=71)	*P* value
Maternal age (year)	27.44±5.11	27.76±5.80	0.30
Maternal BMI (kg/m^2)	28.24±3.87	29.01±4.30	0.38
History of infection during pregnancy (%)	25 (39)	26 (36)	0.93
Smoking	3 (5)	2 (3)	0.77
Gestational age (weeks)	34.30±2.60	39.01±0.97	<0.0001
Delivery method (NVD/CS) (%)	23 (36)/41 (64)	23 (32)/48 (68)	0.67
Neonate gender (male/female) (%)	29 (45)/35 (55)	31 (43)/40 (57)	0.54
Neonatal birth weight (g)	2278.33±655.94	3159.36±359.98	<0.0001

Data are presented as mean±SD or number (%) of subjects, *n*=Number of subjects in each group, BMI=Body mass index, NVD=Natural vaginal delivery, CS=Cesarean section

which had GA genotype. This difference was not statistically significant as well ($P = 0.47$).

Characteristics of mothers and neonates with GA genotype are presented in Table 2.

DISCUSSION

In spite of much effort and extensive research over the past decades, PTD remains an important issue in perinatal care. Depending on the population, PTD may occur in 5-10% of pregnancies and cause serious perinatal morbidity and mortality.[21]

Several observations suggest that women may have a genetic pre-disposition for PTD. Some investigators have reported an association between elevated levels of TNF-α concentration in maternal blood and/or amniotic fluid and PTD.[12,22-25] On the other hand, TNF-α gene production is partly regulated at transcriptional level. The position −308 in TNF-α gene may have a normal guanine (G) allele or have a variant adenine (A) allele.[2]

It has been shown that G308A transition in the promoter region of TNF-α gene results in TNF-α AA or GA genotypes that may induce higher levels of TNF-α gene transcription and activation[20,22] and cause disease susceptibility in the human subjects.[2]

Based on the combination of the above evidence, some investigators have hypothesized that preterm labor may be associated with genetic mutations in TNF-α gene at some positions such as −308.

A number of studies suggest that this genotype variant may affect the likelihood of PTD,[26-30] but some others reject this association.[9,22,31]

Present study demonstrated that despite higher frequency of GA mutation in TNF-α gene at −308 position in mothers and neonates with PTD, this association was not statistically significant. Our findings are consistent with several previous studies, which did not find such association.

Menon *et al.,* performed a meta-analysis of available evidence and concluded that there is no statistically significant association between a SNP in the TNF-α gene PTD.[20]

Dizon-Townson *et al*. also compared the frequencies of TNF T1 or TNF T2 allele between women or fetuses delivered preterm with the control group or previously published allele frequencies and reported that the frequency of TNF-α promoter mutation is not increased in either women or fetuses delivered pretermly.[22]

In addition, *in vitro* assays that used −308 SNP[18,19] and studies that used cells derived from subjects with various TNF-α −308 genotypes showed inconsistent results[33,34] and raised doubts about the functional significance of −308 SNP.[20] The presence of other SNPs in the TNF-α gene and/or variation in other genes that can modify expression, translation and post-translational modification of TNF-α gene could be the probable causes of this condition.[35,36]

A study performed by Moura *et al*. suggests that the combination of TNF-α, including interferon-γ and interleukin-6 maternal gene polymorphisms might contribute to susceptibility to PTD, not the TNF-α polymorphism alone.[3]

Because immunological response is most probably the result of the interaction between several polymorphic genes,[37] it is not be surprising to find a significant association between a single polymorphism and an event such as PTD.

Therefore, it would be logical to investigate the combination of various polymorphisms in different genes to make an appropriate assessment, the role ofpolymorphic genes in the pathogenesis of PTD.[38]

Table 2: Maternal and fetal characteristics of subjects with GA genotype

Maternal and fetal characteristics	TNF-α-308 genotype		Group	Maternal age	Maternal BMI	Gestational age	Delivery method	History of infection during pregnancy	Smoking	Neonatal birth weight	Neonate gender
	Maternal	Fetal									
Case-1	GA	GG	Case	19	25.15	36	NVD	+ (UTI)	-	2200	Female
Case-2	GA	GG	Case	24	24.02	35	NVD	-	-	2350	Female
Case-3	GA	GG	Control	24	26.14	40	NVD	-	-	3200	Female
Case-4	GG	GA	Case	24	22.31	28	c/s	+	-	800	Male

BMI=Body mass index, NVD=Natural vaginal delivery, UTI=Urinary tract infection, GA=Genotyping assays, TNF-α=Tumor necrosis factor alpha

CONCLUSION

In summary, we conclude that there is no significant association between PTD and either maternal or fetal TNF-α −308 polymorphism and frequency of GA mutation is not significantly increased in mothers and neonates delivered preterm. It means that the presence of this mutation by itself does not modify the overall risk of PTD. Investigations on the combination of various polymorphisms in different genes are recommended to achieve more accurate results.

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